#### **REMARKS**

The amendments herein are made to respond to the Notification of Missing
Requirements under 35 U.S.C. 371 in the United States Designated/Elected Office
mailed April 18, 2001 and to better comply with the Sequence Rules. Applicants do not
believe the amendments change the scope of the claims in any way.

In accordance with § 1.825(a) the undersigned hereby states that the paper copy and the computer readable copy of the Sequence Listing supplied herewith are the same, and contain no new matter.

Based on all the foregoing reasons, the sequences now comply with the Sequence Rules. Favorable consideration of this application and early issuance of a Notice of Allowance are most respectfully requested.

RESPECTFULLY SUBMITTED,						
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**Attachments**: Sequence Listing, Paper Copy and Diskette; Marked-Up Copies of Amendments to Specification and Claims.

### Marked-up Copy of Second and Third full Paragraphs on Page 7

Fig. 4 [SEQ. IDs:5-14] [SEQ. ID NOs:5-17]] (AP3, SEQ ID NO:5; DEFA, SEQ ID NO:5; AG\_, SEQ ID NO:6; MCM1, SEQ ID NO:7; SRF, SEQ ID NO:8; GLO, SEQ ID NO:9; RLM1-yeast, SEQ ID NO:10; SMP1-yeast, SEQ ID NO:11; MEF2D, SEQ ID NO:12; AGL5, SEQ ID NO:13; FBP11, SEQ ID NO:14; BOAP1, SEQ ID NO:15; AGL11, SEQ ID NO:16; SPL, SEQ ID NO:17) illustrates the alignment of the first 18 amino acids of the MADS domains from several MADS box transcription factors with amino acids 64 to 80 of the SPL protein.

Fig. 5 [SEQ. ID NO:18] shows the DNA sequence of the promoter of the *SPL* gene and the coding region of the gene. The promoter sequence begins 2690 nucleotides upstream of the start codon of the *SPL* gene. The first nucleotide of the start ATG codon is designated as position +1. The start codon ATG and the stop codon TAA are underlined, and two exons are shown in bold.

#### Marked-up Copy of Paragraph Bridging Pages 7 and 8:

As stated above, the present invention provides isolated nucleic acid molecules (e.g., DNA or RNA) that encode proteins which are involved in, and may be essential to, the formation of meiocytes in the male and female organs of plants. The nucleic acid molecules described herein are useful for producing *Sporocyteless* (SPL) proteins and SPL-type proteins of plant origin when such nucleic acids are incorporated into any of a variety of protein expression systems known to those skilled in the art. An isolated *SPL* gene in accordance with the present invention is shown in Figure 2 [SEQ ID NO:1]. The sequence of the promoter region of the *SPL* gene, as well as the coding region of the gene is shown in Figure 5 [SEQ. ID NO:18].

# Marked-up Copy of Amended First Full Paragraph on Page 16:

There also is provided an isolated nucleic acid sequence or its complement or which hybridizes to said sequence which comprises the contiguous nucleotide sequence as

set forth in Figure 2 [SEQ ID NO:1] or a portion thereof which is preceded by a nucleic acid sequence which provides the promoter region of the gene. A nucleotide sequence which provides the promoter region is shown in Figure 5 [SEQ ID NO:18]. Specifically, the promoter comprises the sequence located within nucleotide positions -2690 to -1 of the sequence set forth in Figure 5 [SEQ ID NO:15] [SEQ ID NO:18], or functional fragments thereof capable of regulating expression of an operably linked gene.

#### Marked-up Copy of Paragraph Bridging Pages 25 and 26:

Another embodiment of the invention provides an isolated promoter of the *SPL* gene. A fragment of DNA extending from 2690 nucleotides upstream of the start codon of the *SPL* gene has been identified as regulating expression of the *SPL* gene. The sequence of this promoter is shown in Figure 5 (SEQ.ID NO: 15) [SEQ. ID NO: 18] as the sequence from base pair -2690 to -1 in the sequence. The first nucleotide of the start ATG codon is designated as position +1 in the sequence. The sequence from -2690 to -1 is sufficient to give *SPL*-specific expression in megasporocytes and microsporocytes. As used herein, "promoter" includes this sequence, a sequence which hybridizes to this sequence and promotes expression of a coding sequence operably linked thereto, and functional fragments of this sequence which are capable of promoting or regulating expression of a coding sequence operably linked thereto. The promoter can be operably linked to a coding sequence if it is linked to the ATG start codon of the coding sequence.

## Marked-up Copy of Paragraph Bridging Pages 31 and 32:

In addition, there is a predicted helix region in SPL protein from amino acids 64 to 85 that has limited homology with the first helix region of the protein motif called the MADS domain that binds DNA. The MADS domain is a highly coserved region of about 57 amino acids found in a family of transcription factors called MADS box factors (See, e.g., Kramer et al., *Genetics* 149:765-783 (1998)). SPL does not have the entire MADS domain, but it shows good conservation to the first 18 amino acids of this domain. A comparison of amino acids 64 to 80 of SPL with the first amino acids of the MADS domain from known regulatory proteins of this class from a variety of species is shown in Figure 4 (SEQ ID NOS:5-18). [SEQ ID NOS:5-17].

### Marked-up Copy of Second Full Paragraph on Page 32:

As shown in Figure 4 [SEQ ID NOS:5-17], the MADS box transcription factors listed are the AP3, AG, AGL5 and AGL11 proteins of Arabidopsis; DEFA and GLO proteins of Antirrhinum (snapdragon); BOAP1 from Brassica oleracea; FBP11 from petunia; MCM1, RLM1, SMP1 proteins from budding yeast; and SRF and MEF2D human proteins.

# Marked-up Copy of Paragraph Bridging Pages 41 and 42:

Two primers, SPL-Xba-S:5'CTAGTCTAGTCTAGAAGATCATCA3' [SEQ ID NO:16] [SEQ ID NO:19] and SPL-BamH1-

- T:5'CGGATCCAAGCTTCAAGGACAAATCAATGGT3' [SEQ ID NO:17] [SEQ ID
- NO:20], which introduced restriction enzyme sites immediately upstream of the *SPL* start codon and the *SPL* stop codon, respectively, were used to amplify the complete *SPL* coding sequence from the cDNA. This amplified fragment was cloned in front of

the GUS gene in the pBI221 vector (Clontech), giving rise to clone pBI221-SPL, which encodes a SPL-GUS fusion. The gene fusion in pBI221-SPL is driven by the 35S promoter and will result in the synthesis in plant cells of a fusion protein consisting of the complete SPL protein at the N terminus and the GUS protein at the C terminus.

### Amended Claims: Version with markings to show changes made

- 45. (Amended) An isolated nucleic acid sequence comprising a nucleic acid sequence as set forth in nucleotides -2690 to -1 of <del>SEQ ID NO. 15</del> SEQ ID NO:18 or a nucleotide sequence which hybridizes to said sequence and promotes expression of a coding sequence operably linked to said nucleotide sequence.
  - 46. (Amended) An isolated nucleotide sequence or functional fragments thereof capable of regulating expression of an operably linked gene, said sequence comprising a nucleotide sequence located within nucleotide positions -2690 to -1 of the nucleotide sequence set forth in SEQ ID NO:18 or a nucleotide sequence which hybridizes to said sequence and promotes expression of an operably linked gene.
  - 47. (Amended) An isolated DNA fragment for directing the expression of a foreign or endogenous gene in a cell, said fragment comprising a sequence as set forth in nucleotides -2690 to -1 of SEQ ID NO:15 SEQ ID NO:18 operably linked to an ATG start codon of a foreign or endogenous gene.
  - 49. (Amended) A method for regulating the expression of a gene which comprises providing a gene of interest operably linked to an *SPL gene* promoter, transferring said operably linked gene to a cell and expressing said gene under gene expression conditions, wherein said *SPL* gene promoter comprises a nucleotide sequence located within nucleotide positions -2690 to -1 of the nucleotide sequence set forth in <del>SEQ ID</del> NO:15 SEQ ID NO:18 or a nucleotide sequence which hybridizes to said sequence and promotes expression of an operably linked gene.